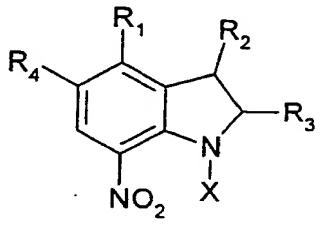
compounds comprising a caging moiety linked to an effector moiety, wherein the compounds are capable of releasing the effector moiety on irradiation, typically by flash irradiation with UV light. The photoreleasable compounds can therefore be used to deliver biologically active effector moieties such as neuroactive amino acids or metal chelators to sites where their activity is required. In preferred embodiments of the invention, the caging moiety is based on 7-nitroindoline and substituted derivatives thereof.

Accordingly, in one aspect, the present invention provides a compound represented by the structural formula:



wherein

R_1 is hydrogen;

C_{1-10} alkyl or substituted alkyl;

$O(CH_2)_n-Y$;

$N(COZ)(CH_2)_mY$; or

$N[(CH_2)_mQ][(CH_2)_nY]$;

R_2 and R_3 are independently selected from:

hydrogen;

C_{1-10} alkyl or substituted alkyl; or

R_2 and R_3 together are cycloalkyl;

R_4 is hydrogen;

C_{1-10} alkyl or substituted alkyl;

phenyl or substituted phenyl;

$(CH_2)_nY$; or

$(CH_2)_mO(CH_2)_nY$;

wherein:

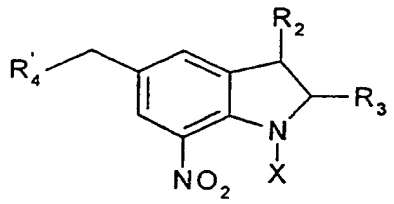
m and n are independently between 1 and 10;

Q and Y are independently selected from hydrogen, CO_2H or salts thereof or OPO_3^{2-};

Z is hydrogen or C_{1-10} alkyl or substituted alkyl; and,

X is an effector moiety or a group capable of being coupled or converted to an effector moiety.

In one embodiment, the present invention provides compounds represented by the structural formula:



wherein

R_2 and R_3 are independently selected from hydrogen, C_{1-10} alkyl or substituted alkyl, or R_2 and R_3 together are cycloalkyl;

R_4' is a blocking group; and,

X is an effector moiety.

The R_4' group blocks the 5-position to ensure that the nitration reaction occurs at the 7-position of the indoline ring. Preferably, R_4' is selected from:

hydrogen;

C_1-C_{10} alkyl or substituted alkyl;

phenyl or substituted phenyl;

$(CH_2)_nCO_2Y$; and,

$(CH_2)_n$-O-$(CH_2)_mY$;

wherein:

m and n are independently between 0 and 10; and,
Y is hydrogen, or C_1-C_{10} alkyl or substituted alkyl.

Exemplary compounds of the invention include:
Methyl 1-glutaryl-7-nitroindoline-5-acetate **8**;
Methyl 1-[(5-dihydroxyphosphoryloxy)pentanoyl)]-7-nitroindoline-5-acetate **9**;
Methyl 1-[*S*-(4-amino-4-carboxybutanoyl)]-7-nitroindoline-5-acetate **10**;
Methyl 1-(4-aminobutanoyl)-7-nitroindoline-5-acetate **21**;
Methyl 1-acetyl-7-nitroindoline-5-acetate **16**;
Mono[1-(5-methoxycarbonylmethyl-7-nitroindolyl)] amide of 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid;
1-Acetyl-4-methoxy-7-nitroindoline **25**;
1-Acetyl-4-methoxy-5-methyl-7-nitroindoline **30**;
1-[*S*-(4-Amino-4-carboxybutanoyl)]-4-methoxy-7-nitroindoline;
1-(4-Aminobutanoyl)-4-methoxy-7-nitroindoline;
1-[(5-Dihydroxyphosphoryloxy)pentanoyl)]-4-methoxy-7-nitroindoline;
Mono[1-(4-methoxy-7-nitroindolyl)] amide of 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid;
1-[*S*-(4-Amino-4-carboxybutanoyl)]-4-methoxy-5-methyl-7-nitroindoline;
1-(4-Aminobutanoyl)-4-methoxy-5-methyl-7-nitroindoline;
1-[(5-Dihydroxyphosphoryloxy)pentanoyl)]-4-methoxy-5-methyl-7-nitroindoline; and
Mono[1-(4-methoxy-5-methyl-7-nitroindolyl)] amide of 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid.

In some embodiments of the invention, the caging moiety is based on substituted 7-nitroindoline. Examples showing the synthesis of substituted 7-nitroindolinyl glutamate and substituted 7-nitroindolinyl GABA, and the

7.97

Methyl 7-nitroindoline-5-acetate 12. A solution of **16** (417 mg, 1.5 mmol) in a mixture of methanol (25 mL), water (5 mL) and conc. HCl (2.5 mL) was heated under reflux for 4 h. The solution was diluted with water (7 mL) and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give a viscous oil. Trituration with ether and recrystallization (Et_2O-hexanes) afforded **12** as red microcrystals (255 mg, 72%), mp 113-115°C; UV: λ_{max} (EtOH)/nm 246 ($\varepsilon/M^{-1}cm^{-1}$ 16 600), 431 (5600); λ_{max} [EtOH-25 mM Na phosphate, pH 7.0 (1:40)]/nm 238 ($\varepsilon/M^{-1}cm^{-1}$ 16 400), 289 (6060), 450 (5060); IR: ν_{max}/cm^{-1} 3420, 3380, 1740, 1645, 1600, 1520; ^1H NMR: δ_H (90 MHz) 7.64 (br s, 1H), 7.16 (br s, 1H), 6.71 (br s, 1H), 3.87 (t, J = 8.3 Hz, 2H), 3.69 (s, 3H), 3.51 (s, 2H) and 3.15 (t, J = 8.3 Hz, 2H). Anal. Calcd for $C_{11}H_{12}N_2O_4$: C, 55.93; H, 5.12; N, 11.85. Found: C, 55.74; H, 5.07; N, 11.68.

A solution of **4** (70 mg, 0.2 mmol) in CH_2Cl_2-dioxane-H_2O (2:3:0.05) (42 mL) was irradiated for 5 h under nitrogen in a Pyrex® flask using a 100 W mercury arc lamp. The progress of photolysis was followed by UV spectroscopy. The solution was concentrated *in vacuo* and the residue was dissolved in EtOAc and washed with saturated aq. $NaHCO_3$ and brine. The organic phase was dried and evaporated and the residue was crystallized (ether-hexanes) to give **12** (44 mg, 95%), mp 113-115°C, identical with material prepared above.

Methyl 7-nitrosoindole-5-acetate 13. A solution of **8** (100 mg, 0.285 mmol) in EtOH (3 mL) was diluted to 60 mL with 50 mM ammonium phosphate, pH 7.0 and irradiated for

5 h under nitrogen in a Pyrex® flask, using a 100 W mercury arc lamp. The progress of photolysis was followed by UV spectroscopy. The combined solutions from two such photolyses were diluted with water and extracted with EtOAc. The combined organic phases were washed with saturated aq. $NaHCO_3$ and brine, dried and evaporated. The residue was flash chromatographed [EtOAc-hexanes (1:4)] to give **13** as green needles (24 mg, 19%), mp 110-111°C (Et_2O-hexanes); UV: λ_{max} (EtOH)/nm 261 ($\varepsilon/M^{-1}cm^{-1}$ 7740), 400 (7260); λ_{max} [EtOH-25 mM Na phosphate, pH 7.0 (1:9)]/nm 278 ($\varepsilon/M^{-1}cm^{-1}$ 6040), 412 (7000); IR: ν_{max}/cm^{-1} ($CHCl_3$) 3460, 1735, 1430, 1395, 1335, 1270, 1180, 1160; ^1H NMR: δ_H (500 MHz) 10.20 (br s, 1H), 9.11 (d, $J_{4,6}$ = 1.3 Hz, 1H, 6-H), 7.97 (d, $J_{4,6}$ = 1.3 Hz, 1H, 4-H), 7.26 (dd, $J_{2,3}$ = 3.2 Hz, $J_{1,2}$ = 3.2 Hz, 1H, 2-H), 6.56 (dd, $J_{2,3}$ = 3.2 Hz, $J_{1,3}$= 2.25 Hz, 1H, 3-H), 4.00 (s, 2H, $ArCH_2$) and 3.76 (s, 3H, OMe); ^{13}C NMR: δ_C (100 MHz) 172.0 (C=O), 155.1 (C-7), 136.2 (C-6), 131.9 (C-4), 131.7 (C-3a or C-7a), 127.9 (C-2), 126.2 (C-5), 116.7 (C-7a or C-3a), 103.1 (C-3), 52.3 (OCH_3) and 40.6 (CH_2). FAB-MS: *m/e* $(M+H)^+$ Calcd for $C_{11}H_{10}N_2O_3$ + H: 219.0770. Found: 219.0762. The ^1H NMR assignments were made from a combination of the 1-dimensional spectrum and nOe experiments, and a COSY spectrum. ^{13}C assignments were made using HSQC and HMBC experiments.

Methyl 1-(5-hydroxypentanoyl)-7-nitroindoline-5-acetate 5. To a solution of the acid **8** (350 mg, 1 mmol) in dry THF (20 mL) at -10°C under nitrogen was added dropwise 1 M BH_3.THF (2 mL, 2 mmol). The mixture was stirred at -10°C for 2.5 h, then quenched with water. The aqueous solution was saturated with K_2CO_3 and extracted with EtOAc. The combined organic phases were washed with brine, dried and evaporated to give a yellow solid.

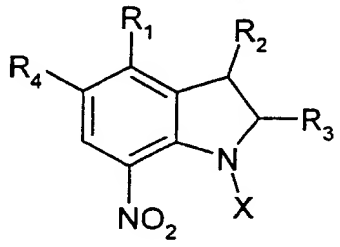
unphotolyzed solution of **18**.

Synthesis of caged GABA. Synthesis of the caged GABA **21** was essentially as described for the glutamate compound **10** (see main text and Supporting Information), starting from the protected derivative **19** (see Experimental Details below). The principal difference was in the isolation protocol, where the properties of the caged GABA required a change of method. Purification of the caged glutamate **10** was effected by preparative HPLC, in which initial elution of the column with aqueous buffer, followed by pure water, sufficed to remove inorganic salts and also separated the small amount of compound **18** that had the hydrolyzed side chain. Elution with water-MeOH (2:1) then gave the pure glutamate compound **10**. In the case of the GABA reagent, which had only one charged group, the compound was significantly more hydrophobic and could not be eluted from a preparative HPLC column using a similar protocol. Instead the compound was eluted from the preparative HPLC column using a mobile phase of aqueous methanolic buffer (see below). Early fractions were contaminated with material assumed to be the hydrolyzed compound **20** but subsequent fractions contained only the required compound **21**. This material was desalted by absorption on Amberlite XAD-2™ resin, that was washed with water to remove buffer salts, then eluted with methanol to recover the caged GABA **21**. As for the caged glutamate **10**, photolysis and quantitative amino acid analysis showed stoichiometric release of GABA.

Methyl 1-(4-aminobutanoyl)-7-nitroindoline-5-acetate 21. Sodium nitrate (93 mg, 1.1 mmol) was added to a stirred solution of **19** (376 mg, 1 mmol) in TFA (5 mL) and the mixture was stirred for 4 h at rt, then evaporated under reduced pressure. The residue was dissolved in water (30 mL) and adjusted to pH 7 with 1 M NaOH. The solution was washed with water, analyzed by reverse-phase HPLC (mobile phase 25 mM Na phosphate, pH 6.0 + 75% MeOH at 1.5 mL/min) and quantified by UV absorption at 342 nm (819 µmol, 82%). HPLC showed major and minor peaks, t_R 6.6 and 1.9 min respectively. The minor peak was assumed to be the free acid **20**. Part of the solution (containing 669 µmol) was lyophilized and purified by preparative HPLC (25 mM Na phosphate, pH 6.0, 2.5 mL/min). The column was first eluted with buffer for 1 h, then with water for 1 h and finally with 10 mM Na phosphate, pH 6.0 + 50% MeOH. Fractions eluted by the last of these eluents were analyzed by reverse-phase HPLC as above. Two early fractions contained both the faster and slower eluting components (total 294 µmol) and were discarded. Subsequent fractions contained only the later-eluting (t_R 6.6 min) component and were combined, quantified by UV absorption (383 µmol) and concentrated under reduced pressure to remove most of the methanol. The residue was diluted to ~20 mL and mixed for 20 min with Amberlite XAD-2™ beads (5 g). The beads were washed with water to remove inorganic salts, then extracted with MeOH (8 x 20 mL). The methanolic solution was quantified by UV (269 µmol), evaporated and the residue containing **21** (phosphate salt) was redissolved in water and stored at -20°C; ^{1}H NMR: δ_H (400 MHz, D_2O, acetone ref.) 7.61 (d, J = 0.7 Hz, 1H), 7.55 (d, J = 0.7 Hz, 1H), 4.32 (t, J = 8 Hz, 2H), 3.83 (s, 2H), 3.72 (s, 3H), 3.25 (t, J = 8 Hz, 2H), 3.08 (t, J = 7.8 Hz, 2H), 2.75 (t, J = 7 Hz, 2H) and 2.02

Claims:

1. A compound represented by the structural formula:



wherein

R_1 is hydrogen;

C_{1-10} alkyl or substituted alkyl;

$O(CH_2)_n$-Y;

$N(COZ)(CH_2)_mY$; or

$N[(CH_2)_mQ][(CH_2)_nY]$;

R_2 and R_3 are independently selected from:

hydrogen;

C_{1-10} alkyl or substituted alkyl; or

R_2 and R_3 together are cycloalkyl;

R_4 is hydrogen;

C_{1-10} alkyl or substituted alkyl;

phenyl or substituted phenyl;

$(CH_2)_nY$; or

$(CH_2)_mO(CH_2)_nY$;

wherein:

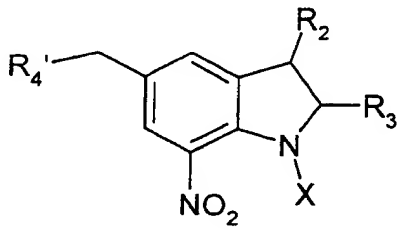
m and n are independently between 1 and 10;

Q and Y are independently selected from hydrogen, CO_2H or salts thereof or OPO_3^{2-};

Z is hydrogen or C_{1-10} alkyl or substituted alkyl; and,

X is an effector moiety or a group capable of being coupled or converted to an effector moeity.

2. The compound of claim 1 represented by the structural formula:



wherein

R_2 and R_3 are independently selected from hydrogen, C_{1-10} alkyl or substituted alkyl, or R_2 and R_3 together are cycloalkyl;

R_4' is a blocking group; and,

X is an effector moiety.

3. The compound of claim 2, wherein R_4' is selected from:

hydrogen;

C_{1-10} alkyl or substituted alkyl;

phenyl or substituted phenyl;

$(CH_2)_nCO_2Y$; and,

$(CH_2)_n-O-(CH_2)_mY$;

wherein:

m and n are independently between 0 and 10; and,

Y is hydrogen, or C_{1-10} alkyl or substituted alkyl.

4. The compound of claim 1 or claim 2 which is:

Methyl 1-glutaryl-7-nitroindoline-5-acetate **8**;

Methyl 1-[(5-dihydroxyphosphoryloxy)pentanoyl)]-7-nitroindoline-5-acetate **9**;

Methyl 1-[*S*-(4-amino-4-carboxybutanoyl)]-7-nitroindoline-5-acetate **10**;

Methyl 1-(4-aminobutanoyl)-7-nitroindoline-5-acetate **21**;
Methyl 1-acetyl-7-nitroindoline-5-acetate **16**;
Mono[1-(5-methoxycarbonylmethyl-7-nitroindolyl)] amide of 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid;
1-Acetyl-4-methoxy-7-nitroindoline **25**;
1-Acetyl-4-methoxy-5-methyl-7-nitroindoline **30**;
1-[*S*-(4-Amino-4-carboxybutanoyl)]-4-methoxy-7-nitroindoline;
1-(4-Aminobutanoyl)-4-methoxy-7-nitroindoline;
1-[(5-Dihydroxyphosphoryloxy)pentanoyl)]-4-methoxy-7-nitroindoline;
Mono[1-(4-methoxy-7-nitroindolyl)] amide of 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid;
1-[*S*-(4-Amino-4-carboxybutanoyl)]-4-methoxy-5-methyl-7-nitroindoline;
1-(4-Aminobutanoyl)-4-methoxy-5-methyl-7-nitroindoline;
1-[(5-Dihydroxyphosphoryloxy)pentanoyl)]-4-methoxy-5-methyl-7-nitroindoline; or,
Mono[1-(4-methoxy-5-methyl-7-nitroindolyl)] amide of 1,2-bis(*O*-aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid.

5. The compound of any one of claims 1 to 4, wherein the effector moiety X is a label, a drug, a toxin, or a carrier or transport molecule.

6. The compound of any one of claims 1 to 5, wherein the effector moiety is an amino acid, a peptide or a polypeptide.

7. The compound of claim 6, wherein the effector moiety is a neuroactive amino acid such as L-glutamate, GABA and glycine.

8. The compound of claim 7, wherein the effector moiety is thyrotrophin releasing hormone, an enkephalin, bradykinin or and angiotensin II.

9. The compound of any one of claims 1 to 4, wherein the effector moiety is metal ion chelator capable of release on photolysis to bind metal ions.

10. The compound of claim 9, wherein the metal ion chelator is EDTA, BAPTA or EGTA.

11. A compound of any one of claims 1 to 10 for use in a method of medical treatment.

12. A compound of any one of claims 1 to 10 for the preparation of a medicament for the treatment of a condition which responds to the effector moiety.

13. A composition comprising a compound of any one of claims 1 to 10.

14. A process for releasing an effector moiety, the process comprising irradiating a photoreleasable compound of any one of claims 1 to 10 to cause the release of the effector moiety.

15. A process for producing a compound of any one of claims 1 to 10, the process comprising:

(a) reacting indoline or a derivatised indoline to substitute a blocking group at the 5-position;

(b) reacting the indoline compound of step (a) to couple an effector moiety at the heterocyclic nitrogen, the effector group having a protecting group; and,

(c) nitrating the indoline compound of step (b) at the 7-position to produce said compound.

16. A process for purifying a compound of any one of claims 1 to 10, the process comprising:

(a) eluting the compound from a HPLC column using aqueous methanol containing buffer salts;

(b) desalting fractions containing the compound obtained from step (a) on Amberlite XAD-2 resin; and,

(c) eluting the resin with methanol to recover the compound.